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DIET AND THE RISK OF INVASIVE CERVICAL CANCER AMONG WHITE WOMEN IN THE UNITED STATES

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A case-control study of incident invasive cervical cancer was conducted in Birmingham, Alabama; Chicago, Illinois; Denver, Colorado; Miami, Florida; and Philadelphia, Pennsylvania, during 1982–1983. Controls were selected by random-digit dialing and were matched to cases by age, race, and telephone exchange. Of the white, non-Hispanic cases and controls identified, 271 (73%) and 502 (74%), respectively, were successfully interviewed. Diet was assessed by asking about the usual adult frequency of consumption of 75 food items and the use of vitamin supplements. Included were the major sources of the four micronutrients believed to reduce the risk of cervical cancer: carotenoids, vitamin A, vitamin C, and folate. Women in the highest quartiles of intake of each of these micronutrients had adjusted relative risks of invasive squamous cell cervical cancer comparable to those of women in the lowest quartiles, although their micronutrient intake was estimated to be 3–4 times as high. Risk was not affected by increased consumption of vegetables, dark green vegetables, dark yellow-orange vegetables, fruits, or legumes, or by high intake of the basic food groups. These generally negative findings stand in contrast to findings in previous epidemiologic studies, and the discrepancy is not readily explained by bias, uncontrolled confounding, or inadequate power. The question of the role of diet and nutrition in the etiology of cervical cancer is not yet resolved.

ascorbic acid; carotene; carotenoids; cervix neoplasms; diet; folic acid; vitamin A; vitamins

A number of epidemiologic studies have suggested that carotenoids, vitamin A, vitamin C, or folate may reduce the risk of cervical cancer (1–16). However, the role of

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Abbreviations: CI, confidence interval; NHANES, National Health and Nutrition Examination Survey.

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diet and nutrition in the etiology of this disease is far from resolved. Many of the relevant epidemiologic studies were of cervical dysplasia, a reversible condition that may or may not lead to cervical cancer (17). A number of the studies identified comparison subjects in a manner substantially different from that used to identify cases, thus potentially biasing the case-control comparisons. Only one study has used community, not hospital, controls (16). Few of the studies collected sufficient information from subjects to adjust dietary findings for potential confounders, and few attempted to evaluate each micronutrient independently.

During 1982–1983, we conducted a large case-control study of incident invasive cervical cancer with community controls in five areas of the United States. The interview used was designed to evaluate all the major sources of carotenoids, vitamin A, vitamin C, and folate in the diet. This paper presents the dietary analysis in non-Hispanic white women. Several other analyses from this study have already been published (18–21).

Materials and methods

This case-control study of invasive cervical cancer was carried out in five US metropolitan areas reporting to the Comprehensive Cancer Patient Data System: Birmingham, Alabama; Chicago, Illinois; Denver, Colorado; Miami, Florida; and Philadelphia, Pennsylvania. Eligible cases were residents of these communities aged 20–74 years who were diagnosed with histologically confirmed primary invasive cancer of the uterine cervix during the period April 1, 1982–December 31, 1983, at one of 24 participating hospitals. Controls from the same communities were selected by random-digit dialing (22, 23). Up to two controls were individually matched to each case on age (±5 years), race (white, Hispanic, black), and telephone exchange (first three digits) (18). Approximately 26 percent of the selected controls had had a hysterectomy and were replaced with others from the control pool. Cases and controls with a history of cancer of other female genital organs were excluded.

The present analysis was restricted to white, non-Hispanic women to eliminate confounding and effect modification by race. It proved difficult to evaluate the role of diet in blacks without knowing how overall etiology was modified by race; racial differences in the risk of invasive cervical cancer are now being analyzed (C. Schairer, National Cancer Institute, personal communication, 1989). Of the cases and controls identified for the study, interviews were successfully completed with 271 (73 percent) and 502 (74 percent), respectively. Subject refusal (10 percent of the cases and 21 percent of the controls) was the major reason for nonparticipation. Other reasons included subject had moved or was not traceable (4 percent and 3 percent, respectively), death (4 percent and 0.4 percent), illness (1 percent and 1 percent), and other problems (0 percent and 1 percent). For 8 percent of the cases, it was not possible to obtain the physician's consent to perform the interview.

The questionnaire was administered to the subject in her home by a trained interviewer. It elicited detailed information on demographic characteristics, sexual behavior, reproductive and menstrual history, use of contraceptives and female hormones, personal and familial medical history, smoking, and diet. Diet was assessed by asking about the "usual adult frequency of consumption, ignoring any recent changes," of 75 food items, listed in the Appendix. Included were the major sources of carotenoids, vitamin A, vitamin C, and folate in the diets of whites, blacks, and Hispanics in the United States, based on

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data from the First and Second National Health and Nutrition Examination Surveys (NHANES I and II) (24, 25). Subjects were asked to respond in terms of number of servings per day, week, month, or year. For seasonal foods, identified in the Appendix, subjects were first asked if the food was eaten mostly in certain seasons or year-round, and then the appropriate frequency of consumption was obtained. Supplementary vitamin intake was assessed by obtaining the duration of use during the last 20 years and the frequency of any supplements taken on a regular basis more than 1 year before the interview. Specifically probed were multivitamins (type and brand were obtained when possible), vitamin A, vitamin C, vitamin E, folic acid, carotene, yeast, wheat germ, and fish liver oil.

The weekly frequency of consumption was derived for each food item. For foods consumed seasonally, an approximate season length was derived from data collected in the US Department of Agriculture Nationwide Food Consumption Survey (26), and the frequency of consumption was time-averaged over a 1-year interval. Food group intake was calculated as the sum of the weekly frequencies of consumption of the food items comprising the food group. Nutrient intake was calculated as the weighted sum of the frequencies of consumption of the food items containing the nutrient; weights used were the nutrient contents of typical servings of the food items. Nutrient intake from vitamin supplements was evaluated separately and was not included in the nutrient indices.

To estimate the nutrient content of a typical serving of a food item, decisions must be made about portion size, food processing and preparation, and food composition tables. For this analysis, portion size and processing and preparation practices (fresh, frozen, or canned; broiled, fried, or sautéed; commercially available or homemade, etc.) were based on the 24-hour dietary recalls collected for a representative sample of the US population in NHANES II during 1976–1980 (25). The frequency distributions seen in the combined 24-hour recalls from women aged 19–74 years were used to calculate the mean nutrient content of the food items in our study questionnaire.

Food composition tables present nutrient content per 100 g for precisely defined food items. The food composition data used in this analysis were, in general, those used in NHANES II, which is a slightly updated version of the values reported in the 1963 US Department of Agriculture's *Composition of Foods* (27). However, we did calculate vitamin A and carotenoid content according to the current convention that dietary carotenoids have one sixth the vitamin A activity of an equivalent weight of dietary retinol (28). Folate content was not available in the NHANES II data base and was obtained from the 1978–1988 US Department of Agriculture's *Composition of Foods* (29), recent laboratory research, and proprietary sources (30).

Food group and nutrient intake were treated as categorical variables. Each measure was stratified into quartiles according to the frequency distribution among the controls in the study population. All races were included in the frequency distribution to permit comparison of race-specific risks for dietary factors.

Ten cases and four controls (1.8 percent of all white subjects) who could not give frequencies of consumption for six or more of the 75 food items were eliminated from the dietary analysis. For the 261 cases and 498 controls remaining, 99.8 percent of the food items had elicited frequencies.

Histologic classification and staging of the cervical cancer diagnoses were based on pathology information from hospital records. Among the 261 cases were 218 squamous cancers, 14 adenosquamous cancers, and 29 adenocarcinomas. The analysis presented in this paper was restricted to the 218 squamous cancers.

The relative risk, as estimated by the odds ratio, was the measure of association used to evaluate the effect of diet on cervical cancer risk. To use information from

more study subjects, we performed unconditional logistic regression to obtain maximum likelihood estimates of the odds ratios and 95 percent confidence intervals, while adjusting for potential confounders (31). Tests for trend were obtained by assigning the score j to the jth level of the dietary exposure and then treating the categorical variable as a continuous variable. Results of the unconditional analyses are presented in this paper. In addition, conditional analyses (32) were performed for the major findings (see tables 1, 2, and 5) and gave similar results.

Potential confounders included number of sex partners, age at first sexual intercourse, years of oral contraceptive use, years since last cervical (Papanicolaou) smear, history of nonspecific gynecologic infection, years of cigarette smoking, usual number of cigarettes/day, years since quitting smoking, education, family income, age at diagnosis, and study center. Full logistic models were run in all analyses, and the number of confounding variables and/or the number of strata of each confounder was gradually reduced in order to approach the simplest model with adequate control of confounding. The relative risks and 95 percent confidence intervals from these simpler models are presented in the text and tables.

Effect modification was evaluated for the same set of variables. Each variable was stratified so that three or, if necessary, two subpopulations were defined, and adjusted relative risks for each subpopulation were derived and compared.

RESULTS

The crude and adjusted relative risks of invasive squamous cell cervical cancer, by quartile of intake of carotenoids, vitamin A, vitamin C, and folate, are shown in table 1. When the adjusted relative risks were examined, no steadily increasing or decreasing risk with decreasing intake was noted for any of the four micronutrients. None of the tests for trend was statistically significant. None of the 95 percent confidence intervals for relative risks in the lowest quartiles of consumption excluded 1.0: for carotenoids, the 95 percent confidence interval was 0.6–1.8; for vitamin A, it was 0.7–2.3; for vitamin C, it was 0.7–2.0; and for folate, it was 0.5–1.5.

The adjusted relative risks of cervical cancer were recalculated by decile of intake of each of these micronutrients. Even when the extreme deciles of micronutrient intake were compared, no clear difference in risk was observed for carotenoids, vitamin A, vitamin C, or folate. Adjusted relative risks were also estimated by quartile of micronutrient intake using the white controls alone as the basis of the quartile cutpoints. Risk was unrelated to intake for each of the four micronutrients. (Recalculations of tables 1 and 2 using quartiles based on the white controls alone are available on request from the authors.)

Table 1 indicates that despite the large number of cervical cancer risk factors potentially associated with dietary patterns, there was little confounding of the relative risks for micronutrient intake in this community-matched case-control study. No single risk factor was primarily responsible for the limited confounding observed. To assess uncontrolled confounding by aspects of life-style not evaluated in the interview, we added education and income to the regression models. The relative risks associated with carotenoid, vitamin A, vitamin C, and folate intake were not altered.

Table 2 presents the adjusted relative risk of invasive squamous cell cervical cancer by intake of basic food groups—fruits, vegetables, legumes, complex carbohydrates, dairy products, and meat and fish. Also included are two vegetable subgroups postulated to reduce cancer risk—dark green and dark yellow-orange vegetables (33)—and two meat and fish subgroups that might indicate the affluence of the diet. For fruit, vegetables, dark green vegetables, dark yellow-orange vegetables, and legumes, no consistent increase or decrease

TABLE 1

Crude and adjusted relative risks of invasive squamous cell cervical cancer, by nutrient intake, among white women in five US areas, 1982–1983*

Nutrient	Quartile of consumption				p_{trend}
	4 (highest)	3	2	1 (lowest)	
Carotenoids					
Crude RR†	1.0	0.67	0.85	1.19	0.18
Adjusted RR	1.0	0.70	0.76	1.02	0.72
n (cases, controls)	(44, 92)	(43, 135)	(57, 141)	(74, 130)	
RE†/day	≥682	468–681	321–467	≤320	
Vitamin A					
Crude RR	1.0	0.82	0.76	0.95	0.98
Adjusted RR	1.0	0.89	0.93	1.25	0.31
n (cases, controls)	(37, 73)	(57, 138)	(54, 141)	(70, 146)	
RE/day	≥1,742	1,095–1,741	732–1,094	≤731	
Vitamin C					
Crude RR	1.0	0.93	1.07	1.43	0.07
Adjusted RR	1.0	1.04	0.88	1.14	0.73
n (cases, controls)	(36, 92)	(45, 124)	(62, 148)	(75, 134)	
mg/day	≥212	146–211	94–145	≤93	
Folate					
Crude RR	1.0	1.01	0.81	0.94	0.59
Adjusted RR	1.0	0.87	0.73	0.85	0.53
n (cases, controls)	(41, 87)	(60, 126)	(56, 147)	(61, 138)	
μg/day	≥303	230–302	172–229	≤171	

* Adjusted for number of sex partners, age at first sexual intercourse, number of cigarettes/day, duration of oral contraceptive use, history of nonspecific genital infection, years since last cervical smear, age at diagnosis, and study center.

† RR, relative risk; RE, retinol equivalents.

in risk with decreasing intake was noted, nor were any of the tests for trend statistically significant. The consumption of dairy products, meat and fish, and expensive and cheap meat and fish was also unrelated to risk. Only complex carbohydrates showed a significant change in relative risk with intake. The relative risk was approximately halved (95 percent confidence interval (CI) 0.3–0.8) in the lowest quartile of complex carbohydrate consumption, compared with the highest quartile. Analogous results were obtained when the adjusted relative risks by food group consumption were recalculated using quartile cutpoints based on the white controls alone.

Our negative findings with respect to intake of carotenoids, vitamin A, vitamin C, folate, and vegetables and fruit are not independent of one another. These micronutrients and this food group were highly intercorrelated in the diets of the white US women in this study. Spearman correlation coefficients ranged from 0.50 for the correlation of vitamin A with vitamin C to 0.82 and 0.80 for the correlations of vegetables and fruit with carotenoids and vitamin C, respectively.

Of particular interest was the influence of folate intake on the risk of cervical cancer in long-term oral contraceptive users, since oral contraceptive use has been proposed to deplete folate in the cervical epithelium (5, 34). Among the 54 cases and 99 controls who had taken oral contraceptives for 5 or more years, there was no evidence of increasing risk with decreasing folate intake. Relative to that in women in the

TABLE 2

Adjusted relative risks (RR) of invasive squamous cell cervical cancer, by food group intake, among white women in five US areas, 1982–1983*

Food group	Quartile of consumption				p_{trend}
	4 (highest)	3	2	1 (lowest)	
Vegetables and fruit					
Adjusted RR	1.0	0.64	0.96	1.11	0.34
Servings/week (27, 28, 30, 34–41, 43, 45, 46, 52–70, 72, 73)†	≥44	31–43	22–30	≤21	
Fruits					
Adjusted RR	1.0	1.00	0.93	1.35	0.26
Servings/week (27, 28, 30, 61–70, 72, 73)	≥19	13–18	7.4–12	≤7.3	
Vegetables					
Adjusted RR	1.0	0.92	1.16	1.16	0.43
Servings/week (34–41, 43, 45, 46, 52–60)	≥26	18–25	12–17	≤11	
Dark green vegetables					
Adjusted RR	1.0	0.98	1.33	1.00	0.69
Servings/week (35, 37, 43, 45, 58)	≥6.3	4.1–6.2	2.5–4.0	≤2.4	
Dark yellow-orange vegetables					
Adjusted RR	1.0	0.89	1.14	1.22	0.32
Servings/week (39, 56, 57)	≥2.5	1.3–2.4	0.55–1.2	≤0.54	
Legumes					
Adjusted RR	1.0	1.04	1.02	0.76	0.36
Servings/week (43–47, 50, 51)	≥7.4	4.7–7.3	2.9–4.6	≤2.8	
Complex carbohydrates					
Adjusted RR	1.0	0.86	0.68	0.45	0.006
Servings/week (12–18)	≥21	15–20	11–14	≤10	
Dairy products					
Adjusted RR	1.0	1.07	0.83	0.90	0.51
Servings/week (22–24, 26)	≥14	8.5–13	4.5–8.4	≤4.4	
Meat and fish					
Adjusted RR	1.0	1.06	0.77	1.05	0.83
Servings/week (1–11)	≥15	12–14	8.8–11	≤8.7	
Expensive meat and fish					
Adjusted RR	1.0	0.93	1.49	1.00	0.65
Servings/week (1–3, 5)	≥8.8	6.6–8.7	5.1–6.5	≤5.0	
Cheap meat and fish					
Adjusted RR	1.0	0.74	0.78	0.77	0.40
Servings/week (4, 6–8)	≥6.5	4.0–6.4	2.3–3.9	≤2.2	

* Adjusted for number of sex partners, age at first sexual intercourse, number of cigarettes/day, duration of oral contraceptive use, history of nonspecific genital infection, years since last cervical smear, age at diagnosis, and study center.

† Numbers in parentheses refer to the Appendix and identify the food items comprising the food group.

highest quartile of folate consumption, the adjusted relative risks among women in the third, second, and first quartiles were 2.2 (95 percent CI 0.5–9.4), 0.7 (95 percent CI 0.1–2.9), and 1.1 (95 percent CI 0.3–4.6), respectively; the p_{trend} was 0.83.

In addition, the relative risks of invasive squamous cell cervical cancer did not vary with intake of carotenoids, vitamin A, vitamin C, or folate among study subjects stratified by age, education, income, number of sex partners, history of nonspecific genital infections, oral contraceptive use, or interval since last cervical smear. However, among heavy smokers (≥21 cigarettes/day), vitamin C intake appeared to be protective, with the relative risk in women in the lowest quartile of intake, relative to those in the highest, reaching 1.9 (95 percent CI 0.5–8.3) (table 3). Among light smokers (1–20 cigarettes/day), risk was slightly elevated among women in the lowest quartile of vitamin C intake, with a relative risk of 1.4 (95 percent CI 0.6–3.4). No elevation of risk with low vitamin C intake was seen among nonsmokers. The risk of cervical cancer also increased with decreasing vitamin C intake among long-term cigarette smokers (≥20 years). Similar elevations in risk with low micronutrient intake among heavy and long-term smokers were noted for folate and vitamin A. Because of correlated variables, it was not possible to determine which micronutrient was the most important or whether heavy smokers or smokers of long duration were the most affected.

Table 4 shows the relative risks of invasive squamous cell cervical cancer by micronutrient intake for early (stage 1) and more advanced (stages 2–4) disease. No clear reduction in risk of early or advanced disease was noted with increased intake of carotenoids, vitamin A, vitamin C, or folate.

Among the controls in this study, 270 (54 percent) had used a vitamin supplement regularly for some period during the last 20 years. A total of 101 (20 percent) had used a multivitamin supplement; 129 (26 percent) had used a multivitamin supplement and at least one other specific supplemental vitamin, not necessarily at the same time; and 40 (8 percent) had used one or more specific supplemental vitamins but no multivitamins. Multivitamin use was associated with reduced risk of invasive squamous cell cervical cancer; the adjusted relative risk for multivitamin use, relative to non-multivitamin use, was 0.85 (95 percent CI 0.6–1.3). As table 5 shows, risk decreased with years of multivitamin use. However, risk was markedly reduced (relative risk = 0.61; 95 percent CI 0.3–1.2) only among women who had taken multivitamins for 15 or more years.

TABLE 3

Adjusted relative risks of invasive squamous cell cervical cancer, by vitamin C intake, among light and heavy cigarette smokers and non-cigarette smokers, among white women in five US areas, 1982–1983*

Smoking intensity	Quartile of vitamin C consumption				p_{trend}
	4 (highest)	3	2	1 (lowest)	
Non-cigarette smokers	1.0	0.90	0.66	0.78	0.46
	(16, 47)†	(18, 69)	(18, 72)	(17, 62)	
Light (1–20 cigarettes/day)	1.0	1.14	0.96	1.41	0.47
	(13, 36)	(19, 40)	(26, 56)	(38, 45)	
Heavy (≥21 cigarettes/day)	1.0	1.03	1.47	1.94	0.27
	(7, 8)	(8, 15)	(18, 20)	(20, 27)	

* Adjusted for number of sex partners, age at first sexual intercourse, duration of oral contraceptive use, history of nonspecific genital infection, and years since last cervical smear.

† Numbers in parentheses, numbers of cases and controls, respectively.

TABLE 4

Adjusted relative risks of early (stage 1) and more advanced (stages 2–4) invasive squamous cell cervical cancer, by nutrient intake, among white women in five US areas, 1982–1983*

Nutrient	Quartile of consumption				p_{trend}
	4 (highest)	3	2	1 (lowest)	
Carotenoids					
Stage 1	1.0 (24)†	0.63 (23)	0.72 (33)	0.72 (32)	0.49
Stages 2–4	1.0 (10)	0.96 (13)	0.78 (15)	0.90 (19)	0.76
Vitamin A					
Stage 1	1.0 (19)	0.91 (29)	0.99 (28)	1.16 (36)	0.54
Stages 2–4	1.0 (8)	1.30 (18)	1.07 (14)	1.42 (17)	0.63
Vitamin C					
Stage 1	1.0 (21)	0.95 (24)	0.63 (30)	0.89 (37)	0.58
Stages 2–4	1.0 (9)	0.90 (11)	0.90 (17)	0.99 (20)	0.97
Folate					
Stage 1	1.0 (22)	0.77 (30)	0.67 (30)	0.70 (30)	0.31
Stages 2–4	1.0 (11)	0.87 (17)	0.55 (15)	0.65 (14)	0.26

* Adjusted for number of sex partners, age at first sexual intercourse, number of cigarettes/day, duration of oral contraceptive use, history of nonspecific genital infection, and years since last cervical smear.

† Numbers in parentheses, number of cases. Among the cases, 112 were diagnosed as stage 1, 41 as stage 2, 13 as stage 3, and 3 as stage 4; 49 were missing this information. The number of controls in each nutrient quartile is given in table 1.

TABLE 5

Adjusted relative risks of invasive squamous cell cervical cancer, by duration of supplemental vitamin use, among white women in five US areas, 1982–1983*

Supplemental vitamin	Years of use					p_{trend}
	0	1–3	4–9	10–14	≥15	
Multivitamins	1.0 (135, 268)†	1.15 (26, 55)	0.92 (25, 64)	0.80 (17, 45)	0.61 (15, 61)	0.15
Vitamin A	1.0 (144, 297)	1.32 (22, 48)	1.00 (21, 57)	0.94 (16, 38)	0.76 (15, 54)	0.51
Vitamin C	1.0 (127, 252)	1.03 (26, 63)	0.94 (25, 65)	0.89 (20, 48)	0.65 (17, 63)	0.24
Folate	1.0 (137, 287)	1.34 (25, 49)	1.19 (25, 60)	0.88 (16, 40)	0.74 (15, 57)	0.52
Vitamin E	1.0 (131, 267)	1.05 (27, 63)	1.07 (26, 64)	0.92 (17, 40)	0.70 (16, 59)	0.40

* Adjusted for number of sex partners, years since last cervical smear, education, and income. Other invasive cervical cancer risk factors were not confounders.

† Numbers in parentheses, numbers of cases and controls, respectively.

Many of the women in the study were able to provide the brand and/or type of multivitamin supplement generally used. Estimates of supplemental vitamin A, vitamin C, folate, and vitamin E intake were derived, based on the content of the multivitamin supplements reported and any use of specific supplemental vitamins. The adjusted relative risks of invasive squamous cell cervical cancer among users of supplemental vitamin A, vitamin C, folate, and vitamin E, relative to the risks among nonusers of the corresponding vitamin, were 0.98, 0.89, 1.03, and 0.94, respectively. The adjusted relative risks by duration of use are shown in table 5. Risk of cervical cancer decreased with years of use of each of these vitamins, but the actual reduction in risk observed among long-term users and the downward trend in risk with duration of

use were not as pronounced as for multivitamin use.

To examine the combined effect of diet and vitamin supplements for vitamin A, vitamin C, and folate, we calculated adjusted relative risks by quartile of dietary micronutrient intake among women who had taken a vitamin supplement containing the micronutrient for more than 3 years and among women who had not (nonusers). Among the nonusers of supplemental vitamin A, vitamin C, or folate, there was no increase in risk of cervical cancer with decreasing dietary intake of the respective micronutrient. In addition, the reduction in risk among users of supplemental vitamin C or folate, relative to that in nonusers of the micronutrient, was observed in women in the highest quartile of dietary intake of the micronutrient, as well as in women in the lowest quartile. Use of supplemental vitamin C was associated with reduced relative risks of 0.70 (95 percent CI 0.3–1.8) and 0.73 (95 percent CI 0.3–1.6), respectively, among women with high and low intake of vitamin C in foods. The analogous relative risks associated with use of supplemental folate, among women with high and low folate intake, were 0.53 (95 percent CI 0.2–1.5) and 0.68 (95 percent CI 0.3–1.7).

Discussion

This case-control study of invasive squamous cell cervical cancer among white US women revealed no evidence of protection by high dietary intake of carotenoids, vitamin A, vitamin C, or folate, although earlier studies of this cancer implicated these micronutrients. Moreover, no reduction in risk was observed with increased consumption of vegetables, dark green vegetables, dark yellow-orange vegetables, fruits, or legumes. Even when the women who had taken micronutrients in vitamin supplements were excluded, cervical cancer risk was unrelated to dietary intake.

The micronutrient levels evaluated in this study were typical of US diets. The exact range of intake over which no change in risk was observed could only be approximated because of problems inherent in estimating absolute intake on the basis of a limited number of food frequencies. However, when the medians in the first and fourth quartiles of consumption were compared, it was apparent that the high consumers in this study population had 3–4 times the intake of the low consumers for each of the four micronutrients. For carotenoids, intake ranged from 236 to 856 retinol equivalents/day; for vitamin A, from 543 to 2,464 retinol equivalents/day; for vitamin C, from 64 to 252 mg/day; and for folate, from 139 to 356 μg/day.

There was no indication in our study that relatively high levels of consumption of the basic food groups characteristic of a well-balanced diet were protective. Thus, generally poor nutrition does not explain why low socioeconomic status persists as a dominant risk factor in our population after adjustment for sexual practices, smoking, and history of cervical smear screening (20).

The difficulty of assessing usual adult dietary patterns in studies of cancer etiology is often emphasized. If micronutrient intake was measured very inaccurately or imprecisely in this study, the resultant estimate could obscure any underlying associations with cervical cancer. However, our study assigned individuals to quartiles of intake and thus did not need to distinguish small differences in diet.

Several lines of evidence suggest that the assessment of micronutrient intake in this study was adequate. First, food frequency interviews comparable to the one used in this study have been shown to give similar results when administered to the same individuals at two different points in time (35–38), to agree well with estimates of nutrient intake based on more detailed food diaries or multiple 24-hour recalls (37–39), and to correlate well with direct measurements of micronutrient levels in serum or plasma (40–42). Each of these methodological approaches partially tests the validity

and reproducibility of a dietary assessment tool. Second, food frequency interviews have been successfully used in other case-control studies to demonstrate associations between nutrient or food group intake and risk of cancer (33, 43, 44). In particular, dietary interviews with this format have consistently demonstrated, in retrospective and prospective studies among different populations, inverse associations between risk of lung cancer and vegetable and fruit and carotenoid intake (45). Thus, inaccurate or imprecise measurement of micronutrient intake does not seem to explain our negative results.

Power calculations indicate that, with a two-sided significance level of 0.05, this study would have had an 80 percent probability of detecting a relative risk of 1.65 among women in the lowest quartile of micronutrient intake and a 98 percent probability of detecting a relative risk of 2.0. Thus, it is not likely that this study failed to find a substantial association with diet by chance alone.

Theoretically, bias might have prevented detection of an association with micronutrient intake. If carotenoids, vitamin A, vitamin C, or folate were indeed protective, then the cases would have had to recall eating *more* of the micronutrient than they actually consumed for bias to obscure the underlying association. It is generally postulated that cancer patients recall eating *less* because of loss of appetite due to disease. In this study, we saw no clear difference in the micronutrient intake reported by women with invasive cervical cancer at an early stage and that reported by women with more advanced disease, which suggests that dietary recall was not biased by the presence of cancer. In addition, there was no evidence that the cases remembered eating more or less of the basic food groups than the controls remembered. Thus, recall bias does not seem to explain our negative findings.

Another potential source of bias in this study was differential participation. Although net participation rates among eligible cases and controls were similar (73 percent and 74 percent, respectively), a larger number of controls than cases actually refused participation (21 percent vs. 10 percent, respectively). It is conceivable that the controls of lower socioeconomic status (and more limited diets) would have been less willing to answer questions for a health survey. However, this situation would have generated the appearance of reduced micronutrient intake among the cases. Thus, differential participation does not easily explain the absence of any protective effects.

At least 16 papers have been published on the role of diet and nutrition in the etiology of cervical cancer (1–16). Eight of the studies evaluated dietary intake of micronutrients (1–3, 6–9, 16); 10 of them examined micronutrient levels in serum or plasma (4, 5, 7, 10–16). All but two of these studies (4, 13) found that levels of at least one micronutrient (carotenoids, beta-carotene, vitamin A, vitamin C, or folate) were lower in women with cervical disease (cervical dysplasia, cervical carcinoma in situ, or invasive cervical cancer) than in comparison individuals. Of all these studies, three (8, 12, 16) are notable because they identified controls comparable to the cases and adjusted for cervical cancer risk factors in analysis.

La Vecchia et al. (8), in a hospital-based case-control study of invasive cervical cancer with 191 cases and 191 controls conducted in Milan, Italy, showed marked increases in risk (relative risks of approximately 5) with decreased intake of green vegetables and carrots. In a study conducted in Oxford, England, Harris et al. (12) measured serum beta-carotene in 32 women with invasive cervical cancer, 81 women with in situ cervical cancer or cervical dysplasia, and a control group of 226 women with benign gynecologic problems. Compared with women in the highest quartile, women in the lowest quartile of serum beta-carotene levels had approximately

three times the risk of preinvasive disease but showed no increased risk of invasive disease. Brock et al. (16), in a case-control study of in situ cervical cancer with 117 cases and 196 community controls conducted in Sydney, Australia, found that risk of in situ disease increased with decreasing intake of beta-carotene, vitamin C, and folate and with decreasing plasma beta-carotene levels. Micronutrient intake was based on an extensive list of food frequency questions that included approximate portion size. Thus, our negative findings about the role of micronutrients in the etiology of cervical cancer do not concur with the findings in the published literature.

Evidence that folate is involved in the etiology of cervical cancer derives primarily from a randomized trial conducted by Butterworth et al. (5). Forty-seven women with mild or moderate cervical dysplasia who were using oral contraceptives were given 10 mg of supplemental folic acid (25 times the Recommended Dietary Allowance (28)) or a placebo daily. It has been proposed that continued use of oral contraceptive steroids alters folate metabolism in the cervical epithelium and induces a localized folate deficiency (34), thus predisposing the cervix to neoplastic changes. During the 3-month trial, the severity of dysplasia steadily decreased among the women who were using folate, while the status of the placebo group was unchanged (5). In our study, there was no indication that high dietary intake of folate altered cervical cancer risk in oral contraceptive users. However, the levels of intake in our study would not have reached the therapeutic doses administered in the randomized trial.

One positive finding of our study was a possible protective effect of vitamin C in heavy cigarette smokers (≥21 cigarettes/day). This finding is provocative and deserves to be pursued in future studies. It is consistent with the ability of vitamin C to capture free radicals in cigarette smoke and prevent oxidative degradation of cellular structures (46) and with the demonstration in hamster lung cell cultures that vitamin C reverses cigarette smoke-induced malignant changes (47). However, this subgroup observation was one of many examined and may be due to chance.

Another positive finding of our study was a reduction in risk of cervical cancer with years of multivitamin use; however, there are several reasons to be skeptical about this result. First, the reduction in risk became less pronounced when the actual content of the most commonly used multivitamin was considered. Second, a clear reduction in risk, 0.7–0.8 the risk in nonusers, was seen only in women who had used supplemental vitamin A, vitamin C, folate, or vitamin E for 15 or more years. Third, no analogous protective effect was noted for intake of micronutrients in food. Fourth, the reduction in risk associated with supplemental vitamin C or folate use was noted in women with relatively high, as well as relatively low, dietary intake of the respective micronutrient. It is possible that long-term supplemental vitamin users adhered to other aspects of life-style, possibly involving sexual, contraceptive, or smoking practices, that reduced their risk of cervical cancer. However, it is conceivable that only the women using supplemental vitamins attained high enough serum micronutrient levels to affect carcinogenesis.

For a number of the women in the study, the information on the type of multivitamin supplement generally used was not sufficiently specific or clear to identify its exact content, and reasonable assumptions had to be made. Thus, it is possible that differential recall could have biased the results for supplemental vitamin A, vitamin C, folate, and vitamin E.

In summary, the risk of invasive squamous cell cervical cancer was not reduced by high dietary intake of carotenoids, vitamin A, vitamin C, or folate in our community-based case-control study in white US women. Our negative findings do not agree with much of the published epi-

demiologic literature. The reason is not clear, but the findings cannot be easily attributed to imprecise measurement of diet, recall bias, selection bias, differential participation, inadequate control of confounding, or low statistical power. It is noteworthy, however, that our study is the only case-control study of invasive cervical cancer with community controls. Other studies have investigated in situ cervical cancer (16) or cervical dysplasia (2, 5), demonstrated associations for preinvasive disease alone (12), and/or used hospital controls (2, 6, 8, 12). Our inability to find evidence of protection by carotenoids, vitamin A, vitamin C, or folate introduces a note of caution into the dietary intervention studies planned for this cancer and leaves the question of the role of micronutrients in the etiology of cervical cancer unresolved.

References

1. Hirayama T. Diet and cancer. Nutr Cancer 1979; 1:67–81.
2. Wassertheil-Smoller S, Romney SL, Wylie-Rosett J, et al. Dietary vitamin C and uterine cervical dysplasia. Am J Epidemiol 1981;114:714–24.
3. Romney SL, Palan PR, Duttagupta C. Retinoids and the prevention of cervical dysplasias. Am J Obstet Gynecol 1981;141:890–4.
4. Lambert B, Brisson G, Bielmann P. Plasma vitamin A and precancerous lesions of cervix uteri: a preliminary report. Gynecol Oncol 1981;11:136–9.
5. Butterworth CE Jr, Hatch KD, Gore H, et al. Improvement in cervical dysplasia associated with folic acid therapy in users of oral contraceptives. Am J Clin Nutr 1982;35:73–82.
6. Marshall JR, Graham S, Byers T, et al. Diet and smoking in the epidemiology of cancer of the cervix. J Natl Cancer Inst 1983;70:847–51.
7. Bernstein A, Harris B. The relationship of dietary and serum vitamin A to the occurrence of cervical intraepithelial neoplasia in sexually active women. Am J Obstet Gynecol 1984;148:309–12.
8. La Vecchia C, Franceschi S, Decarli A, et al. Dietary vitamin A and the risk of invasive cervical cancer. Int J Cancer 1984;34:319–22.
9. Wylie-Rosett JA, Romney SL, Slagle NS, et al. Influence of vitamin A on cervical dysplasia and carcinoma in situ. Nutr Cancer 1984;6:49–57.
10. Orr JW Jr, Wilson K, Bodiford C, et al. Nutritional status of patients with untreated cervical cancer. II. Vitamin assessment. Am J Obstet Gynecol 1985;151:632–5.
11. Romney DL, Duttagupta C, Basu J, et al. Plasma vitamin C and uterine cervical dysplasia. Am J Obstet Gynecol 1985;151:976–80.
12. Harris RWC, Forman D, Doll R, et al. Cancer of the cervix uteri and vitamin A. Br J Cancer 1986;53:653–9.
13. Heinonen PK, Kuoppala T, Koskinen T, et al. Serum vitamins A and E and carotene in patients with gynecologic cancer. Arch Gynecol Obstet 1987;241:151–6.
14. Palan PR, Romney SL, Mikhail M, et al. Decreased plasma beta-carotene levels in women with uterine cervical dysplasias and cancer. J Natl Cancer Inst 1988;80:454–5.
15. Romney SL, Basu J, Vermund S, et al. Plasma reduced and total ascorbic acid in human uterine cervix dysplasias and cancer. Ann NY Acad Sci 1987;498:132–43.
16. Brock KE, Berry J, Mock PA, et al. Nutrients in diet and plasma and risk of in situ cervical cancer. J Natl Cancer Inst 1988;80:580–5.
17. Stern E, Neely PM. Carcinoma and dysplasia of the cervix: a comparison of rates for new and returning populations. Acta Cytol 1963;7:357–61.
18. Brinton LA, Schairer C, Haenszel W, et al. Cigarette smoking and invasive cervical cancer. JAMA 1986;255:3265–9.
19. Brinton LA, Huggins GR, Lehman HF, et al. Long-term use of oral contraceptives and risk of invasive cervical cancer. Int J Cancer 1986; 38:339–44.
20. Brinton LA, Tashima KT, Lehman HF, et al. Epidemiology of cervical cancer by cell type. Cancer Res 1987;47:1706–11.
21. Brinton LA, Hamman RF, Huggins JR, et al. Sexual and reproductive risk factors for invasive squamous cell cervical cancer. J Natl Cancer Inst 1987;79:23–30.
22. Waksberg J. Sampling methods of random digit dialing. J Am Stat Assoc 1978;73:40–6.
23. Hartge P, Brinton LA, Rosenthal JF, et al. Random digit dialing in selecting a population-based control group. Am J Epidemiol 1984;120:825–33.
24. National Center for Health Statistics. Plan and operation of the Health and Nutrition Examination Survey, United States, 1971–1973. Hyattsville, MD: US Department of Health, Education, and Welfare, 1973. (Vital and Health Statistics: programs and collection procedures. Series 1, no. 10a). (DHEW publication no. (HSM)73-1310).
25. National Center for Health Statistics. Plan and operation of the second National Health and Nutrition Examination Survey, 1976–1980. Hyattsville, MD: US Department of Health and Human Services, 1981. (Vital and Health Statistics: programs and collection procedures. Series 1, no. 15). (DHHS publication no. (PHS)81-1317).
26. US Department of Agriculture. Food consumption: households in the Northeast, North Central Region, South, and West, seasons and years, 1977–78. Nationwide Food Consumption Survey 1977–78. Hyattsville, MD: US GPO, 1983. (Reports no. H-7, 8, 9, 10).
27. US Department of Agriculture. Composition of foods. (USDA handbook no. 8). Washington, DC: US Department of Agriculture, 1963.
28. National Academy of Sciences. Recommended Dietary Allowances. 9th rev. ed. Washington, DC: National Academy of Sciences, 1980.

29. US Department of Agriculture. Composition of foods. (USDA handbooks no. 8-1 to 8-16). Washington, DC: US Department of Agriculture, 1976–1986.
30. Subar AF, Block G, James LD. Folate intake and food sources in the U.S. population. Am J Clin Nutr 1989;50:508–16.
31. Prentice RL, Pyke R. Logistic disease incidence models and case-control studies. Biometrika 1979;66:408–12.
32. Lubin J. A computer program for the analysis of matched case-control studies. Comput Biomed Res 1981;14:138–43.
33. Ziegler RG, Mason TJ, Stemhagen A, et al. Carotenoid intake, vegetables, and the risk of lung cancer among white men in New Jersey. Am J Epidemiol 1986;123:1080–93.
34. Whitehead N, Reyner F, Lindenbaum J. Megaloblastic changes in the cervical epithelium: association with oral contraceptive therapy and reversal with folic acid. JAMA 1973;226:1421–4.
35. Rohan TE, Potter JD. Retrospective assessment of dietary intake. Am J Epidemiol 1984;120:876–87.
36. Willett WC, Sampson L, Stampfer MJ, et al. Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol 1985;122:51–65.
37. Sampson L. Food frequency questionnaires as a research instrument. Clin Nutr 1985;4:171–8.
38. Pietinen P, Hartman AM, Haapa E, et al. Reproducibility and validity of dietary assessment instruments. II. A qualitative food frequency questionnaire. Am J Epidemiol 1988;128:667–76.
39. Willett WC, Sampson L, Browne ML, et al. The use of a self-administered questionnaire to assess diet four years in the past. Am J Epidemiol 1988;127:188–99.
40. Willett WC, Stampfer MJ, Underwood BA, et al. Validation of a dietary questionnaire with plasma carotenoid and alpha-tocopherol levels. Am J Clin Nutr 1983;38:631–9.
41. Russell-Briefel R, Bates MW, Kuller LH. The relationship of plasma carotenoids to health and biochemical factors in middle-aged men. Am J Epidemiol 1985;122:741–9.
42. Stryker WS, Kaplan LA, Stein EA, et al. The relation of diet, cigarette smoking, and alcohol consumption to plasma beta-carotene and alpha-tocopherol levels. Am J Epidemiol 1988;127:283–96.
43. Ziegler RG, Morris LE, Blot WJ, et al. Esophageal cancer among black men in Washington, D.C. II. Role of nutrition. J Natl Cancer Inst 1981; 67:1199–206.
44. McLaughlin JK, Gridley G, Block G, et al. Dietary factors in oral and pharyngeal cancer. J Natl Cancer Inst 1988;80:1237–43.
45. Ziegler RG. A review of epidemiologic evidence that carotenoids reduce the risk of cancer. J Nutr 1989;119:116–22.
46. Ames BN. Dietary carcinogens and anticarcinogens. Science 1983;221:1256–64.
47. Leuchtenberger C, Leuchtenberger R. Protection of hamster lung cultures by L-cysteine or vitamin C against carcinogenic effects of fresh smoke from tobacco or marihuana cigarettes. Br J Exp Pathol 1977;58:625–34.

APPENDIX

Food items in a diet questionnaire administered to women in five US areas, 1982–1983

Nonseasonal	Seasonal*
1. Beef	27. Orange juice
2. Chicken or turkey	28. Grapefruit juice
3. Pork	29. Vitamin C-fortified fruit drink or juice
4. Combination dishes with some meat, such as spaghetti and meatballs	30. Acerola juice
5. Fish, fresh or frozen	31. Beer
6. Canned fish	32. Wine
7. Cold cuts or luncheon meats	33. Hard liquor
8. Hot dogs or franks	34. Iceberg or head lettuce
9. Beef or calves' liver	35. Leaf lettuce
10. Chicken or pork liver	36. Cabbage or cole slaw
11. Liverwurst or liver sausage	37. Greens
12. Whole wheat or cracked wheat bread or rolls	38. Beets
13. White, rye, or pumpernickel bread or rolls	39. Carrots
14. Biscuits, muffins, or flour tortillas	40. Celery
15. Corn bread, corn tortillas, corn fritters, or corn pudding	41. Sweet green peppers
16. White or brown rice	42. Red chili peppers or red sweet peppers
17. Noodles, macaroni, or spaghetti	43. Green peas
18. White potatoes	44. Black-eyed peas, crowder peas, chick-peas, or dried peas
19. Cake, cookies, pies, or cupcakes	45. Green beans, string beans, or pole beans
20. Pancakes, waffles, doughnuts, sweet rolls, or coffee cake	46. Lima beans
21. Eggs	47. All other beans, such as pinto beans and frijoles
22. Cottage cheese or yogurt	48. Tomato sauce, cooked tomatoes, or tomato soup
23. Cheese, including such dishes as macaroni and cheese	49. Vegetable soup or a stew made with vegetables
24. Ice cream	50. Nuts
25. Butter or margarine	51. Peanut butter
26. Milk	52. Cucumbers
	53. Corn
	54. Raw tomatoes
	55. Summar squash
	56. Pumpkin or winter squash
	57. Sweet potatoes or yellow yams
	58. Broccoli
	59. Cauliflower
	60. Asparagus
	61. Strawberries
	62. Apricots
	63. Cantaloupe
	64. Watermelon
	65. Peaches or nectarines
	66. Apples
	67. Bananas or plantains
	68. Pears
	69. Oranges or tangerines
	70. Grapefruit
	71. Avocado or guacamole
	72. Guava
	73. Papaya or mango
	74. Hot cereals
	75. Cold cereals

* For these foods, the respondent was asked whether the food was eaten mostly in certain seasons, year-round, or not at all.